Long-term effect of preventive therapy for tuberculosis in a cohort of HIV-infected Zambian adults

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Objective: To determine the long-term effect of preventive therapy (PT) for tuberculosis on the rates of tuberculosis, mortality and HIV progression.

Methods: In a randomized controlled trial, 1053 HIV-positive Zambian adults received isoniazid (H) for 6 months, rifampicin plus pyrazinamide (RZ) for 3 months, or a placebo. CD4 percentage, neopterin, absolute lymphocyte count and haemoglobin were measured from enrolment (absolute CD4 cell counts from 12 months after enrolment). Because PT reduced the incidence of tuberculosis, eligible placebo subjects were offered H. Here, tuberculosis and mortality rates are compared in the three original arms (intention to treat) using data beyond the end of the trial (average follow-up 3 years; maximum 7 years).

Results: There were 102 cases of tuberculosis and 281 deaths (rates 3.6 and 9.0/100 person-years, respectively). There was no significant difference between the tuberculosis rates in the H and RZ groups at any time. The effect of H/RZ on tuberculosis diminished over time ($P = 0.011$) but the cumulative risk of tuberculosis in the first 2.5 years was significantly lower in the H/RZ group than the placebo group (rate ratio 0.55; 95% confidence interval 0.32–0.93; $P = 0.028$). There was no significant effect of PT on mortality or progression markers. Tuberculosis was associated with an increased mortality (adjusted rate ratio 1.96; 95% confidence interval 1.21–3.18; $P = 0.006$).

Conclusions: Both PT regimens protect against tuberculosis for at least 2.5 years but appear to have no effect on HIV progression or mortality. These results may be used in cost-effectiveness models of PT.

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Tuberculosis, HIV, survival, progression, preventive therapy, durability, Africa

Introduction

The World Health Organization estimates that in 1999 there were 33.6 million people infected with HIV worldwide and about a third of these were infected with *Mycobacterium tuberculosis* [1]. HIV infection is the strongest risk factor for both reactivation of latent tuberculosis and for progression from primary infection with *M. tuberculosis*. Latent tuberculosis may be treated with preventive therapy (PT) and this has been recommended as a means of preventing tuberculosis in HIV-infected subjects [2].

Six randomized controlled trials have shown that PT for tuberculosis reduces the incidence of tuberculosis in HIV-infected adults [3–8], although another trial showed no significant effect [9]. Two recent meta-analyses have assessed the overall efficacy of PT on the incidence of tuberculosis and mortality in HIV-infected adults [10,11]. Both analyses showed that PT for 3–12

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months protects against tuberculosis and that the effect is strongest in those with a positive tuberculin skin test (TST). The effect of PT on HIV progression and mortality is less clear. Neither the individual trials nor the meta-analyses showed a significant effect of PT on mortality. One trial [3] reported the effect of PT on progression to AIDS and found a significant effect only in the 63 TST-positive subjects. None of the trials reported the effect of PT on HIV progression markers.

In the absence of HIV infection, PT has been shown to give substantial long-term protection against tuberculosis [12]. However, the durability of the effect of PT on tuberculosis and mortality in HIV-infected subjects has not been assessed, since none of the trials had a long duration of follow-up (the mean ranged from 0.3 to 3.1 years). Data on the durability of the effect of PT on tuberculosis and mortality are needed to estimate the number of subjects needed to be treated to prevent one case of tuberculosis, the extended life expectancy of persons living with HIV and, hence, the cost-effectiveness of PT [13,14]. Here we report the long-term effect of PT for tuberculosis on the rates of tuberculosis, mortality and HIV progression in a cohort of 974 Zambian adults. We also describe the effect of tuberculosis on mortality. The cohort comprised subjects enrolled into a Zambian trial of PT [8]. Subjects were followed beyond the end of the trial and, to date, the accumulated follow-up averages 3 years per person, with a maximum follow-up of 7 years.

Methods

Enrolment and follow-up

Between August 1992 and June 1994, 1053 HIV-infected adults were enrolled into a randomized controlled trial of PT for tuberculosis. Details of the trial methods and results are published elsewhere [8]. The trial was conducted at the University Teaching Hospital in Lusaka and received ethical approval from the Ethics Committee of the University of Zambia and the Ministry of Health, and the London School of Hygiene and Tropical Medicine. Subjects were eligible for inclusion in the trial if they were HIV positive, aged 15 years or older and gave written consent. Patients were excluded if they had a previous history of treatment for tuberculosis, had active tuberculosis, had abnormal liver function tests, were pregnant or lived too far away from the study clinic. Patients in the trial were randomly assigned to receive 900 mg of isoniazid (H) twice weekly for 6 months, 600 mg rifampicin (rifampin) plus 3500 mg pyrazinamide (RZ) twice weekly for 3 months or a placebo that matched H twice weekly for 6 months, or a placebo that matched RZ twice weekly for 3 months. Subjects were seen routinely in the clinic every month for the first 6 months and every 3 months thereafter. The study clinic also provided open access to those with intercurrent problems.

The trial outcomes were the incidence of tuberculosis and mortality. Tuberculosis was diagnosed if there was a positive smear or culture for *M. tuberculosis* or positive histology. Tuberculosis was also diagnosed if there were pulmonary infiltrates and clinical symptoms, no response to two broad-spectrum antibiotics and a positive response to tuberculosis treatment within 2 months. Some subjects had respiratory symptoms and radiological features suggestive of tuberculosis and no positive sputum results; these patients were started on tuberculosis treatment without completing two courses of antibiotics because of the severity of their clinical condition. They are defined as 'probable tuberculosis' provided that they had a positive response to tuberculosis treatment within 2 months. Date of death was ascertained from hospital notes or by interviewing relatives. The trial data were analysed in April–July 1996 and showed that H and, to a lesser extent, RZ were associated with a lower tuberculosis incidence rate. In August 1996, 112 subjects taking placebo (72% of the subjects in this group who had not developed tuberculosis or died and who were still attending the study clinic) were given H for 6 months. The remaining 44 placebo subjects refused H, had possible active tuberculosis or were not eligible (e.g. pregnancy, abnormal liver function tests). Subjects continued to attend the clinic every 3 months and have been followed up until death or censored at September 1999.

Progression markers

A venous blood sample was requested at every second routine visit (i.e., every 6 months). A full blood count was performed and absolute CD4 and CD8 cell counts were estimated using a FACScount analyser (Becton Dickinson, Mountain View, California, USA) with controls and reagents supplied by the manufacturer. In addition, blood smears were prepared as for conventional haematological examination and sera were stored at −20°C. The frozen blood smears were stored with desiccant for up to 3 months until the immunocytochemical labelling was performed with monoclonal antibodies against CD4 and CD8 receptors (Dako, Copenhagen, Denmark) using the immunoalkaline phosphatase technique. CD4 and CD8 percentages were determined by counting 200 lymphocytes on each slide. Serum neopterin levels were measured using radioimmunosorbant assay (RIA; BRAHMS, Berlin, Germany) with controls supplied by the manufacturer. CD4 percentages were measured in 606 subjects (62%) in the first 2 years of follow-up. Neopterin counts were measured in 835 subjects (86%) in the first 3 years of follow-up. CD4 cell counts were measured in 575 subjects (59%) from month 12–18 until the end of the study. Lymphocyte counts and haemoglobin levels

were measured in 91% of subjects throughout the study.

Statistical methods

Tuberculosis and mortality were analysed separately; follow-up was slightly longer for the analysis of mortality. The follow-up data were censored at September 1999. The primary analysis compares tuberculosis and mortality rates in the three original trial arms (intention to treat) even though some of the subjects in the placebo group received H in August 1996. The incidence of tuberculosis in the placebo group, therefore, may underestimate the incidence rate in a 'pure' placebo group if 'delayed H' (i.e. started in August 1996) protected against tuberculosis. The H and RZ groups were analysed separately and in combination (H/RZ). However, the results were similar when the analysis was performed using the pure placebo group (censored at July 1996) as the comparison group and using different definitions of tuberculosis (data not shown). All rate ratios (RR) are crude unless stated otherwise. Most RR values were not adjusted for potential confounders because the three arms were similar with respect to enrolment characteristics. RR and 95% confidence intervals (CI) were estimated using Cox regression, and statistical significance of RR was assessed using the likelihood ratio test. A change in the effect of PT on tuberculosis or mortality over time was assessed using an interaction test (P value denoted as P_i), which tested for a linear change in log RR over time (i.e., increasing or decreasing RR over time). The effect of tuberculosis on mortality was assessed in a Cox model with tuberculosis treated as a time-dependent exposure.

For each subject, the annual change in each progression marker over time was estimated using (last count − first count)/follow-up time. For many subjects, the annual change was estimated over 6 months of follow-up for CD4 cell percentage, over 2 years of follow-up for CD4 cell count, CD8 cell count and neopterin and over 30 months of follow-up for lymphocyte count and haemoglobin. The annual change per subject was also estimated from the slope of the linear regression of the progression marker against follow-up time. Here, linear regression was performed on transformed data where the original data did not have a normal distribution. The square root transformation was used to normalize CD4 cell count, and natural logarithms were used to normalize neopterin and lymphocyte count. For subjects with three or more values of a progression marker, this approach utilized all the serial data and hence provided a more accurate estimate of change. The median annual change in subjects in each PT group was compared with the median in the placebo group using the Wilcoxon rank sum test. All data analysis was performed using Stata version 6 (Stata Corporation, East College Station, Texas, 77840, USA).

Results

Enrolment and follow-up

Figure 1 shows the profile of the trial participants. The placebo, H and RZ groups were similar at enrolment with respect to age, sex, marital status, education, crowding, BCG scar, TST status, enlarged epitrochlear lymph nodes, oral candidiasis and herpes zoster [8]. The RZ group had a slightly lower lymphocyte count and haemoglobin level at enrolment but these differences were not statistically significant. By September 1999, the 1053 subjects in the study had been followed for 3138 person-years (mean 3.0), which was an additional 1368 person-years compared with the original trial analysis. The total follow-up for tuberculosis was 2800 person-years (mean 2.7), an additional 1169 person-years compared with the original trial. Table 1 shows the enrolment characteristics of subjects with complete ($\geq$ 3 years) and incomplete follow-up. The number of subjects with incomplete follow-up because of losses or death is similar in the three groups ($P = 0.26$) although the RZ group has slightly less subjects with $\geq$ 3 years of follow-up. No follow-up is associated with being female, young, less educated and recruited at George Clinic (a primary health care clinic in one of the high-density residential areas). The 79 subjects with no follow-up were excluded from the remaining analysis (Fig. 1).

Effect of preventive therapy on tuberculosis

During the course of the study, there were 102 cases of tuberculosis, 74 of which were culture, smear or histopathologically positive. In addition, there were 47 cases of probable tuberculosis. The overall incidence rate was 3.6/100 person-years for tuberculosis and 5.3/100 person-years for tuberculosis/probable tuberculosis. The incidence of tuberculosis was lower in the H and

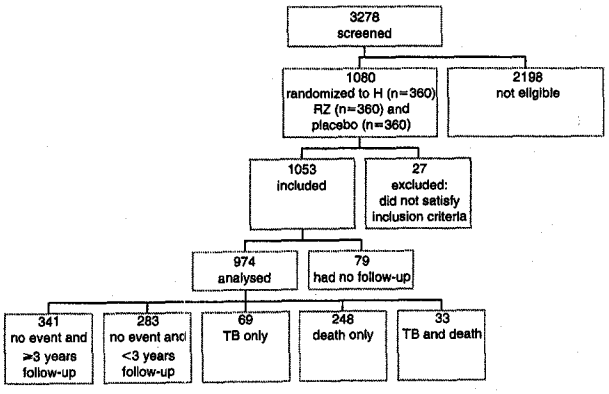

Fig. 1. Trial profile.

Table 1. Enrolment characteristics of subjects according to follow-up status.

Variable	0	Percentage at follow up (years) of < 1.5 Died	< 1.5 Lost	1.5–3.0 Died	1.5–3.0 Lost	⩾ 3.0
Number	79	112	171	92	130	469
PT group						
Placebo	28	29	36	33	32	35
H	26	34	36	28	34	34
RZ	46	38	35	39	35	31
Sex						
Male	47	58	53	53	55	61
Female	53	42	47	47	45	39
Age (years)						
< 25	42	19	26	16	37	23
25–39	55	64	66	63	48	64
⩾ 40	3	17	9	21	15	13
Marital status						
Married	51	59	62	61	62	62
Single	31	19	24	13	25	26
Widowed/divorced	18	22	14	26	14	12
Education (years)						
⩾8	39	52	53	51	59	58
1–7	57	43	42	42	37	38
None	4	4	5	7	4	4
Clinic						
George	66	47	51	48	48	45
Other	34	53	49	52	52	55

PT, preventative therapy; H, isoniazid; RZ, rifampicin plus pyrazinamide.

RZ groups than in the placebo group censored at July 1996 or the placebo group censored at September 1999 (Table 2). Among the cases of tuberculosis that occurred after July 1996 in the placebo group, 11 were in the group who received H from August 1996 (incidence rate 4.0/100 person-years) and one was in the group who never started H (incidence rate 2.1/100 person-years).

Figure 2 shows survival until tuberculosis in the three arms. Both PT regimens are associated with a lower incidence of tuberculosis, but the effect diminishes over time; the interaction between PT and time is statistically significant for H, RZ and H/RZ (Tables 3 and 4). Despite the diminishing RR at each time interval beyond 1.5 years, the cumulative risk of tuberculosis in the first 2.5 years was lower in the H group (RR 0.52; 95% CI 0.27–1.00; $P = 0.046$), the RZ group (RR 0.58; 95% CI 0.30–1.09; $P = 0.082$) and the H/RZ group (RR 0.55; 95% CI 0.32-0.93; $P = 0.028$) compared with the placebo group. The incidence of tuberculosis tended to be higher in the RZ group than in the H group, although this effect was not statistically significant at any time interval and did not change over time (Table 4).

The TST status of patients at enrolment was available for 678 patients, of whom 161 (24%) were TST positive. The effect of PT in TST-positive subjects was stronger than in all subjects, particularly during the first

Table 2. Rates of tuberculosis and mortality by preventive therapy group.

Group (period[a])	Subjects	Follow-up[b] [median years (range)]	Tuberculosis [No./patient-years (rate)])	Mortality [No./patient-years (rate)]
Placebo (1)	350	2.1 (0–3.9)	28/617 (4.5)	64/692 (9.2)
Placebo then no H (2)	44	0.3 (0–3.3)	1/48 (2.1)	11/81 (13.6)
Placebo then H (2)	112	2.7 (0.1–3.3)	11/274 (4.0)	15/296 (5.1)
Intention to treat				
Placebo (1+2)	350	2.1 (0–6.9)	40/940 (4.3)	90/1068 (8.4)
H (1+2)	352	2.2 (0–7.1)	29/957 (3.0)	85/1061 (8.0)
RZ (1+2)	351	2.1 (0–6.9)	33/903 (3.7)	106/1009 (10.5)

H, isoniazid; RZ, rifampicin plus pyrazinamide.
[a]Period 1, data censored at July 1996; period 2, data starting after July 1996; period 1+2, data censored at September 1999.
[b]Figures are for follow-up until tuberculosis (follow-up for mortality is slightly longer).

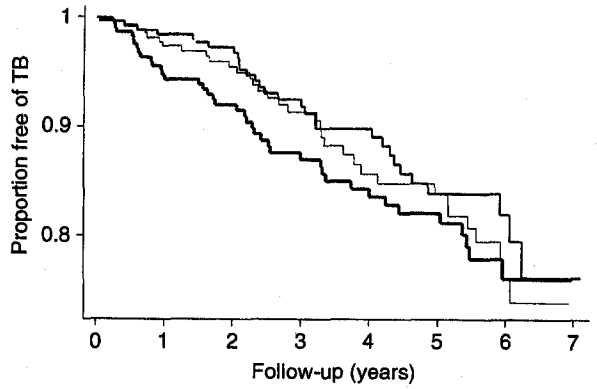

Fig. 2. Kaplan–Meier curves showing survival until tuberculosis in patients treated with placebo (–), isoniazid (–) and rifampicin plus pyrazinamide (–).

1.5 years (Table 4). Despite the small numbers contributing to each time-specific RR, there was strong evidence of diminishing RR over time. Nevertheless, compared with the placebo group, the cumulative risk of tuberculosis in the first 2.5 years was substantially lower in the H/RZ group (RR 0.32; 95% CI 0.16–0.67; $P = 0.002$). Total lymphocyte count was measured at enrolment in 858 subjects, of whom 740 (86%) had a count $\geq 1.5 \times 10^9$ cells/l. In these 740 subjects, the effect of PT diminished over time (Table 4) and, compared with the placebo group, the RR for tuberculosis during the first 2.5 years was 0.54 (95% CI 0.31–0.95; $P = 0.033$) for the H/RZ group.

Effect of preventive therapy on mortality

There were 281 deaths during the study period, resulting in a mortality rate of 9.0/100 person-years. Figure 3 shows survival until death in the three arms. The mortality rate was slightly higher in the RZ group than in the H or placebo groups (Table 2). Compared with the placebo group, the RR for death was 0.95 (95% CI 0.71–1.28; $P = 0.75$) in the H group and 1.24 (95% CI 0.94–1.65; $P = 0.13$) in the RZ group. The higher mortality rate in the RZ group compared with the placebo and H groups was observed in all time intervals but was not statistically significant (Table 3). After adjusting for age and sex, the overall and time-specific RR values for RZ were reduced (data not shown). There was no significant change in the effect of PT on mortality over time in either all subjects (Table 4) or the TST-positive subjects ($P_I = 0.38$ for H/RZ versus placebo).

Effect of preventive therapy on HIV progression markers

Table 5 shows the median annual change in progression markers in the three arms for subjects with two or more values recorded, and the median follow-up time over which each progression marker was measured. For all progression markers except CD4 percentage, at least 75% of subjects who had two or more values recorded also had three or more values recorded. The RZ group tended to have a slower decline in lymphocyte count than the placebo group but, in general, there was no effect of H or RZ on any of the progression markers. The results were similar for comparisons between the mean annual change (of transformed data where appropriate) using the t-test (data not shown).

Effect of tuberculosis on mortality

The effect of tuberculosis on mortality was assessed in a Cox model with tuberculosis as a time-dependent exposure, allowing for the potential confounding effects of age, sex, PT group and enrolment progression markers (either neopterin or lymphocyte count since CD4 count was not measured at enrolment). Tuberculosis was associated with a twofold increase in mortality (neopterin adjusted RR 1.96; 95% CI 1.21–3.18; $P = 0.006$ and lymphocyte adjusted RR 2.83; 95% CI 1.83–4.38; $P < 0.001$). The effect of tuberculosis on mortality did not vary according to PT group ($P_I = 0.78$ for interaction). The median survival time

Table 3. Rates of tuberculosis and mortality stratified by follow-up time.

	Rate by years of follow-up [No./patient-years (rate)]		
Group	< 1.5	1.5–2.5	> 2.5
Tuberculosis			
Placebo	15/372 (4.0)	11/181 (6.1)	14/387 (3.6)
H	5/377 (1.3)	9/191 (4.7)	15/389 (3.9)
RZ	7/369 (1.9)	8/182 (4.4)	18/352 (5.1)
H/RZ	12/745 (1.6)	17/373 (4.6)	33/741 (4.5)
Mortality			
Placebo	32/406 (7.9)	22/209 (10.5)	36/451 (8.0)
H	38/407 (9.3)	20/210 (9.5)	27/442 (6.1)
RZ	41/395 (10.4)	27/205 (13.2)	38/406 (9.4)
H/RZ	79/802 (9.8)	47/415 (11.3)	65/848 (7.7)

H, isoniazid; RZ, rifampicin plus pyrazinamide.

Table 4. Rate ratios for tuberculosis and mortality stratified by follow-up time.

Event	Rate ratios (95% CI) at follow-up (years)			*P* value
	< 1.5	1.5–2.5	> 2.5	
Tuberculosis in all subjects				
Placebo	1	1	1	
H	0.33 (0.12–0.91)	0.78 (0.32–1.87)	1.07 (0.52–2.23)	0.045
RZ	0.46 (0.19–1.14)	0.72 (0.29–1.79)	1.39 (0.69–2.79)	0.031
H/RZ	0.40 (0.19–0.85)	0.75 (0.35–1.60)	1.22 (0.65–2.29)	0.011
RZ versus H	1.43 (0.45–4.50)	0.93 (0.36–2.41)	1.30 (0.65–2.58)	0.72
Tuberculosis in 161 subjects with a positive TST				
Placebo	1	1	1	
H	0.16 (0.04–0.71)	0.86 (0.26–2.82)	1.23 (0.49–3.12)	0.020
RZ	0.31 (0.10–0.97)	0.17 (0.02–1.42)	1.15 (0.47–2.84)	0.053
H/RZ	0.24 (0.09–0.62)	0.51 (0.17–1.59)	1.19 (0.54–2.63)	0.009
Tuberculosis in 740 subjects with lymphocyte at month 0 of $\geq 1.5 \times 10^9$/l				
Placebo	1	1	1	
H	0.31 (0.10–0.96)	0.71 (0.29–1.77)	1.00 (0.46–2.16)	0.043
RZ	0.54 (0.22–1.35)	0.64 (0.25–1.64)	1.20 (0.57–2.52)	0.078
H/RZ	0.43 (0.19–0.95)	0.67 (0.31–1.47)	1.10 (0.57–2.12)	0.018
Mortality in all subjects				
Placebo	1	1	1	
H	1.18 (0.74–1.89)	0.91 (0.49–1.66)	0.77 (0.47–1.27)	0.22
RZ	1.31 (0.83–2.09)	1.25 (0.71–2.19)	1.18 (0.75–1.86)	0.74
H/RZ	1.25 (0.83–1.88)	1.07 (0.65–1.78)	0.97 (0.64–1.45)	0.39
RZ versus H	1.11 (0.72–1.73)	1.37 (0.77–2.44)	1.54 (0.94–2.52)	0.33

CI, confidence interval; H, isoniazid; RZ, rifampicin plus pyrazinamide; TST, tuberculin skin test.
All rate ratios compare preventive therapy with placebo unless stated otherwise.
P value for test of interaction between preventative treatment and follow-up time.

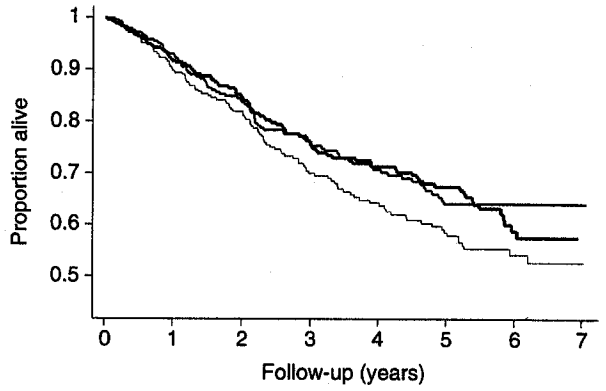

Fig. 3. Kaplan–Meier curves showing survival until mortality in patients treated with placebo (–), isoniazid (–) and rifampicin plus pyrazinamide (–).

from diagnosis of tuberculosis was 33 months and the 1-, 2- and 3-year survival percentages were 80, 65 and 46%, respectively. Probable tuberculosis was associated with a worse prognosis than tuberculosis. The median survival time from diagnosis of tuberculosis/probable tuberculosis was 25 months and the 1-, 2- and 3-year survival percentages were 72, 52 and 38%, respectively. The mortality rate from diagnosis of tuberculosis was 33/146 = 22.6/100 person-years in those with tuberculosis, and 28/38 = 73.7/100 person-years in those with probable tuberculosis.

Discussion

The extended follow-up of this cohort allowed us to assess the long-term effect of two alternative PT regimens on tuberculosis, mortality and HIV progression. Twice weekly H taken for 6 months and twice weekly RZ taken for 3 months both protect against the incidence of tuberculosis. These effects diminish significantly over time but the cumulative effect is evident 2.5 years after the start of PT. There was no significant difference between the incidence of tuberculosis in the H and RZ groups at any time interval. Tuberculosis was associated with a twofold increase in mortality. Despite observing a protective effect of PT on tuberculosis and a higher mortality associated with tuberculosis, we were unable to demonstrate a significant effect of PT on mortality or on any of the HIV progression markers studied.

The present study is based on a large number of subjects with a relatively long follow-up. The study subjects are from an area with a high prevalence of HIV infection, a high incidence of tuberculosis and where antiretroviral drugs are hardly accessible. Participants were included irrespective of TST status or CD4 cell count. The main limitation of the study arises from potential biases from the loss of patients during follow-up. There was no follow-up for 79 subjects (7.5%) and a further 171 (16%) had less than 1.5 years of follow-up. The losses were similar in the three arms (Table 1)

Table 5. Median annual change in progression markers in preventive therapy and placebo groups.

	No. subjects	No. (%) with ≥ 2 values	Median follow-up (months)	Annual charge (arithmetic)[a]		Annual change (linear regression)[b]	
				Median (IQR)	*P* value[c]	Median	*P* value[c]
CD4 cell ($\times 10^6$ cells/l)							
Placebo	350	166 (47)	27	−28.1 (−63 to −12)	–	−1.19	
H	352	164 (47)	26	−28.4 (−64 to −8)	0.67	−1.10	0.79
RZ	351	146 (42)	21	−33.5 (−65 to −9)	0.79	−1.10	0.92
CD4 cell (%)							
Placebo	350	106 (30)	6	−2.0 (−11 to 8)		−2.0	
H	352	107 (30)	6	−2.4 (−11 to 8)	0.99	−2.4	0.95
RZ	351	96 (27)	6	−4.0 (−15 to 7)	0.26	−4.0	0.25
Neopterin							
Placebo	350	232 (66)	23	1.57 (−0.79 to 5.83)		0.13	
H	352	223 (63)	23	0.97 (−0.99 to 5.00)	0.32	0.08	0.27
RZ	351	217 (62)	23	0.84 (−1.72 to 4.95)	0.12	0.06	0.11
Lymphocyte							
Placebo	350	268 (77)	38	−0.07 (−0.35 to 0.15)		−0.036	
H	352	254 (72)	30	−0.10 (−0.38 to 0.20)	0.45	−0.054	0.24
RZ	351	254 (72)	28	−0.04 (−0.25 to 0.32)	0.066	−0.006	0.033
Haemoglobin							
Placebo	350	266 (76)	38	−0.19 (−0.64 to 0.22)		−0.17	
H	352	254 (72)	32	−0.18 (−0.64 to 0.18)	0.83	−0.14	0.63
RZ	351	255 (73)	28	−0.20 (−0.68 to −0.24)	0.65	−0.18	0.83

IQR, interquartile range; H, isoniazid; RZ, rifampicin plus pyrazinamide.
[a]Annual change estimated as (last value − first value)/follow-up.
[b]Annual change estimated from linear regression using the following transformations: square root for CD4 cells (so median represents median annual change in square root CD4 cells); natural logarithm for neopterin; natural logarithm for lymphocyte count.
[c]*P* value for Wilcoxon rank sum test for difference between preventive therapy group and placebo.

so any selection bias is likely to be non-differential and this may result in the effect of PT being underestimated. Serial progression marker measurements (two or more values) were available for a large number of subjects (ranging from 309 for CD4% to 776 for lymphocyte count), thus allowing the estimation of the effect of PT on changes in progression markers. A substantial proportion of subjects, however, had less than 2 values recorded (ranging from 71% for CD4% to 26% for lymphocyte count and haemoglobin). Again, there were no substantial differences in these proportions between the three arms, but groups of subjects such as those who died will be under-represented.

The protective effect of PT on the incidence of tuberculosis confirms the findings of the meta-analyses [10,11], and the similarity between the effects of H and RZ confirms the findings of another trial [15]. Prior to the HIV epidemic, the efficacy of PT was shown to be of long duration, possibly even lifelong [12]. The diminishing effect of PT over time in HIV-infected Zambian adults may be explained by the high risk of new infection or by inadequate sterilization of dormant tubercle bacilli in the absence of immunity. We examined whether the diminishing effect of PT over time arose because some of the original placebo group started a 6-month course of H in August 1996, and this may have offered protection against tuberculosis thereafter. However, the diminishing effect of PT over time was statistically significant when we censored the data at July 1996 and used the pure placebo group as the comparison. We observed similar results on the efficacy of PT on tuberculosis when we used different definitions of tuberculosis.

An important finding of the present study is the absence of protection of PT on HIV progression, using either laboratory markers or mortality as an endpoint. Moreover, for all analyses, the RZ group had a higher mortality rate than the H and placebo groups. Confounding is the most likely explanation for this effect. The RZ group tended to have a lower lymphocyte count and haemoglobin level at enrolment, and adjusting for these factors, together with age and sex, reduced the effect of RZ on mortality. Four of the five trials of PT that stratified by TST status found a lower mortality rate in TST-positive subjects taking PT compared with those taking placebo [3,5,7,9] although this effect was not statistically significant in any trial or in the meta-analyses [10,11]. We do not know the exact proportion of deaths in the present study that were

related to tuberculosis. Many of the deaths occurred at home and, therefore, accurate data on cause of death were not available. Epidemiological data on the effect of tuberculosis on HIV progression markers are scarce [16]. We also failed to demonstrate an effect of PT on HIV progression, though the effect of PT on mortality and progression markers in our study may be underestimated because of the missing data.

The subjects who developed tuberculosis in the present study had twice the mortality rate of those who remained free of tuberculosis. This is consistent with the findings of two other studies that observed an excess mortality associated with tuberculosis, adjusting for CD4 cell count and previous opportunistic infections [17,18]. However, tuberculosis has been associated with a lower mortality rate compared with other AIDS-defining conditions [19]. Clearly, the comparison group used for assessing the effect of tuberculosis must be taken into account when interpreting these results [16]. The median survival from diagnosis of tuberculosis in the present study (33 months) and the 1- and 3-year survival proportions (80% and 46%, respectively) are consistent with those found in HIV-infected tuberculosis patients in the United States [17], France [18] and a previous Zambian study [20].

In conclusion, twice weekly H taken for 6 months and twice weekly RZ taken for 3 months both protect against the incidence of tuberculosis for at least 2.5 years. These effects are not confined to TST-positive subjects; consequently, screening for TST positivity may not be a necessary component of implementation of PT. The PT regimens studied neither slowed HIV progression nor protected against mortality. Our estimates of the durability of the effect of PT on tuberculosis and mortality may be used in cost-effectiveness models of PT. Additional trials are required to assess the efficacy and cost-effectiveness of longer regimens of PT, while further epidemiological studies may help to elucidate the effect of tuberculosis on HIV progression.

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